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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/423,554	11/10/1999	ARISTOS ARISTIDOU	0933-148P	6884

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EXAMINER	
WALICKA, MALGORZATA A	
ART UNIT	**PAPER NUMBER**
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/423,554	ARISTIDOU ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 February 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *25,26,43,45,46,49,57,60,61 and 68* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *25 and 26* is/are allowed.

6)☒ Claim(s) *43,45,46,49,57,60,61 and 68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

The Reply under 37 CFR § 1.116 filed on Feb. 1, 2005 comprising amendments to the claims and specification is acknowledged. The finality of the last Office Action is withdrawn. Claims 1-24 and 27- 42, 44, 47, 48, 50-56, 58, and 62-65 have been previously canceled; claims 59 and 66 are currently canceled. Claims 43, 45 and 57 have been amended. Claims 25, 26, and 43, 45, 46, 49, 57, 60, 61, 67 and 68 are pending and are the subject of this Office Action.

Detailed Office Action

1. Constructive election

In the Remarks of Feb. 1, 2005 the Attorney traversed the constructive election and requested its withdrawal. The ground for withdrawal of the constructive election presented on page 14, line 3 is as follows:

> "Applicants respectfully submit that the products recited in claims 43 and 57 all relate to a single inventive concept under PCT Rule 13.1 in that industrial production of all of these products in a microorganism results in either depletion of NAD/NADPH cofactors or an increase in NADH/NADP cofactors. Therefore, the claims of the present invention possess unity of invention under PCT rules."

Applicants' argument has been fully considered but is found not persuasive for the following reasons. Increasing the fermentor production of ethanol, xylitol, lysine and other amino acids wherein the specific transformant is used has continued to be a subject of articles and patents in the biotechnology for many years. In the Information Disclosure Statement Applicants enclosed article by Meinander et al., (A heterologous

reductase affects the redox balance of recombinant Saccharomyces cerevisiae, Microbiology, 1996, 142, 165-172) in which the authors strongly suggest:

"The addition of the second [to the xylose reductase] enzyme necessary for complete xylose metabolism in S. cerevisiae, XDH [xylose dehydrogenase], **could in part reduce these effects by regenerating the NADH consumed in the XR catalyzed reaction** [emphasis added]. However, the fact remains that XR seems to prefer NADPH over NADH in anoxic conditions *in vivo*, and therefore some of the effects on the redox balance are likely to remain even after the introduction of XDH", page 171, left column, third paragraph.

Other authors disclosed in Information Disclosure Statement anticipated what Meinander et al. suggested and obtained S. cerevisiae transformants containing XR and XDH genes: Hallborn et al., EP 0527758 (WO91/1558, published 17/10, 1991) and Koetter et al. (Isolation and characterization of the Pichia stipitis xylitol dehydrogenase gene, XYL2, and construction of a Xulose-utilizing Saccharomyces cerevisiae transformant, Current Genetics, 1990, 18, 493-500). Thus, the introduction of a dehydrogenase to balance the concentration of cofactors NAD and NADPH is not a contribution over the prior art and the claims as originally filed and currently presented are missing a special technical features.

In addition, as explained in the restriction requirement of 04/05/2001, the claims are drawn to four independent methods of making different chemical compounds. 37 CFR 1.475 does not provide for multiple products or **methods** within a single application and therefore unity of invention is lacking with regard to Groups I-IV.

In summary, the restriction requirement of April 5, 2001 is proper and the request of constructive election as issued in the Office Action of October 1, 2004 is proper as well. All the currently presented claims are considered only in relation to production of ethanol, as originally elected and thus far examined.

2. Objections

The objections to claim 43 and 57 for minor informalities are withdrawn, because the claims have been amended.

3. REJECTIONS

3.1. 35 USC 112, second paragraph

Claims 43 and 57 were rejected for recitation the term "a microorganism" in the 6th and 9th line. This rejection is now withdrawn, because the claims have been amended.

3.2. 35 USC 112, first paragraph

3.2.1. Lack of written description

Rejection of claims 48, 59 and 66 made in the Office Action of Oct. 1, 2004 is moot because the claims have been canceled.

Claim 43, 45, 46, 49, and 61 and claims 57, 60, 67 and 68-68 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description

requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to a method of increasing the yield of production of ethanol by fermenting yeast cell transformant, wherein the method comprises as the first step transforming any yeast cell with one or more polynucleotides encoding an enzyme selected from the group consisting of:

glutamate dehydrogenase,

malic enzyme,

aldehyde dehydrogense,

malate dehydrogenase,

glycerol-phosphate dehydrogenase,

xylose-1-dehydrogenase,

glyceraldehyde-3-phosphate dehydrogenase, and

orotate reductase.

The claims are directed to large and versatile genus of methods using a large and versatile genus of host cells of any yeast species transformed to express one or more of eight exogenous dehydrogenases listed above. The specification lacks of sufficient written description of the genus of transformants encompassed by the broad scope of the claims because Applicants disclose only several useful transformants. Applicants' basic yeast cells that are used as control in examining the yield of ethanol

production are integrants comprising xylose reductase (XR) and xylitol dehydrogenase (XGH) genes of *Pichia stipitis* (*XYL1* and *XYL2*); see Example 1. The integrant are identified as

1) H1346 (VTT C—98304) and

2) H1469 (VW-1B).

For the purpose of the present analysis the abbreviation of the name of exogenous enzymes will be used after Applicants name for each integrant which is subsequently further engineered to obtained more complex transformants. Thus, basic integrants are

1) H1346 (S. cerevisiae + XR +XDH) and

2) H1469 (S. cerevisiae + XR +XDH).

Glutamate dehydrogenase

Integrants 1 and 2 are transformed with genes encoding xylulokinase of S.cerevisiae (XK) and/or glutamate dehydrogenase, NAD dependent, encoded by *GDH2* gene, in a single copy (GDH) or multicopies (mGDH): see Examples 2 and 3.

Applicants has shown the **increase in ethanol production related to introduction of glutamate dehydrogenase gene** for **two transformants,**

H1803 (S. cerevisiae + XR + XDH + XK + **GDH**), Fig. 4, and

H1791 (S. cerevisiae + XR + XDH + **mGDH**), Table 1,

as compared to their controls (S. cerevisiae + XR +XDH +XK) and (S. cerevisiae + XR + XDH).

Malic enzyme

Applicants also teach **three transformants** of S. cerevisiae transformed with **malic enzyme (ME)** having higher production of ethanol than the transformant, which does not overexpress ME. Trasformant H2193 (S. cerevisiae + XR + XDH + mME) as compared to (S. cerevisiae + XR + XDH), growing on glucose (Table 3) xylose and transformant H2195 (S. cerevisiae + XR + XDH), growing on xylose; see Fig. 9. However, the statistical significance of the measurement is uncertain in this case. In case of H2222 (S. cerevisiae + XR + XDH + XK +m **ME**) growing on xylose, the increase in production of ethanol, Fig. 10, and was substantial.

Applicants also teach S. pombe transformant H2369 (S. pombe +XR+XDH +mME) which produces more alcohol than (S. pombe +XR+XDH); see Fig. 14.

Providing evidence that two transformants of S. cerevisiae transformed with particular set of genes and GDH or mGDH, as well as three transformants of S. cerevisiae comprising the same set of genes plus ME gene, and providing evidence that one S. pombe transformant having particular set of genes plus ME gene produce more ethanol than their not transformed counterparts, does not provide identifying characteristics of all transformants of any yeast species that, because they were transfected with any dehydrogenase or reductase listed in the claims, produce more ethanol than the parent cells. The specification is silent of any transformant comprising aldehyde dehydrogense, malate dehydrogenase, glycerol-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, orotate reductase and ferrodixin

reductase and producing ethanol with higher yield than the counterpart cells not being transformed with these genes. Applicants even do not provide any evidence that transforming a yeast cell comprising XR with XDH will increases production of ethanol. Thus, the specification is lacking written description of any yeast cell transformed with one or more polynucleotide encoding an enzyme selected from the group consisting of:

glutamate dehydrogenase,

malic enzyme,

aldehyde dehydrogense,

malate dehydrogenase,

glycerol-phosphate dehydrogenase,

xylose-1-dehydrogenase,

glyceraldehyde-3-phosphate dehydrogenase,

orotate reductase and

ferrodoxin reductase,

wherein said transformant produces more ethanol than its parental cell.

In conclusion, claim(s) 43, 45, 46, 49, and 61 and claims 57, 60, 67 and 68-68 contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had the possession of the claimed invention.

In addition, Applicants' attention is turned to the fact that the production of ethanol is dependent on fermentation conditions and genetic set of transformants used

for fermentation. Those skilled in the art realize that overexpression of glutamate dehydrogenase may lead to decrease in ethanol production; see, for example, Lincino Da Silva et al. 1992. Those skilled in the art also know that overexpressing of glycerol 3-phosphate dehydrogenase results in drop of ethanol production; see for example Eglington J. M. et al, Yeast, 2002, and deletion of glycerol 3-phosphate dehydrogenase gene causes an increase in ethanol production (Valadi et al. 1998), which is the opposite of that what one would expect reading the specification. Deleting NADPH-dependent glutamate dehydrogenase, which is glutamate dehydrogenase, as recited in claims 43 and 57, causes not a decrease but and increase in ethanol production, which is the opposite of that what one wold expect reading Applicants disclosure; see Nissen T. L. et al. 2000. All articles mentioned in this comment are enclosed in examiner's references, PTO Form 982.

Furthermore most of dehydrogenases as Applicants disclose, are in forms that are NAD or NADP dependent and the result of transformation will be a combination of the intricacies of the metabolism of the yeast cell to be transformed and the form of dehydrogenase chosen.

Claim 57-68 is additionally rejected for lack of written description of

1) genus of substrate X
2) genus of substrate Y
3) genus of substrate Z
4) genus of enzymes that are referred to as Enzyme 4 in cycle 2, which is said not be a dehydrogenase.

The scope of the claim broad and Applicants do not teach which substrate belonging to genera 1-4 are to be included or excluded from the scope of the claim.

In conclusion claim(s) 57, 60, 67 and 68-68 contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had the possession of the claimed invention.

Claim 46 and 68 are rejected, because they are directed to method of increasing production of ethanol from any carbohydrate, whereas Applicants disclose production of ethanol from glucose or xylose, thus the broad scope of the genus of carbohydrates that can be used for production of ethanol is not sufficiently described. Applicants do not disclose production of ethanol from, for example, cellulose or starch. Thus, claim(s) 46 and 68 contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had the possession of the claimed invention.

3.2.2. Scope of enablement

Claim 43, 45, 46, 49, and 61 and claims 57, 60, 67 and 68-68 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for transforming yeast cells with any genes encoding

a) glutamate dehydrogenase,

b) malic enzyme,

c) aldehyde dehydrogense,

d) malate dehydrogenase,

e) glycerol-phosphate dehydrogenase,

f) xylose-1-dehydrogenase,

g) glyceraldehyde-3-phosphate dehydrogenase,

h) orotate reductase and

i) ferrodixin reductase,

as well as being enabling for transformants H1803 (S. cerevisiae + XR + XDH + XK + **GDH**), H1791 (S. cerevisiae + XR + XDH + **mGDH**), H2195 (S. cerevisiae + XR + XDH + **mME**), H2222 (S. cerevisiae + XR + XDH + XK +**mME**) and H2369 (S. pombe +XR+XDH +**mME**) that produce more ethanol than their counterparts not comprising GDH or ME, does not reasonably provide enablement for any yeast cell transformed with gene encoding any of the enzymes a) – j), and reactions performed in cycle 1 and 2 in claims 57-68, wherein said transformant produces more ethanol than its counterpart that does not comprise gene encoding any of the enzymes a) – j). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention.

Factors to be considered in determining whether undue experimentation is required are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir.

1988)]. The Wands factors are: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance presented, (c) the presence or absence of working example, (d) the nature of the invention, (e) the state of the prior art, (f) the relative skill of those in the art, (g) the predictability or unpredictability of the art, and (h) the breadth of the claim.

The nature and breadth of the claimed invention encompasses transformation of any yeast cell, i.e. any species of yeast, wild type, naturally occurring mutants as well as any mutants and transformants man-made with a gene selected from genes encoding

a) glutamate dehydrogenase,

b) malic enzyme,

c) aldehyde dehydrogense,

d) malate dehydrogenase,

e) glycerol-phosphate dehydrogenase,

f) xylose-1-dehydrogenase,

g) glyceraldehyde-3-phosphate dehydrogenase,

h) orotate reductase and

i) ferrodixin reductase,

so as the resulting transformant produced more ethanol than its not transformed parental cell and wherein in addition in claims 57, 60 and 67-68 the enzymes a)-j) perform reactions of cycle 1 or 2 with any

1) substrate X

2) substrate Y

3) substrate Z, in cooperation with

4) any enzyme 4 in cycle 2, which is said not be a dehydrogenase.

Although the art of genetic engineering of yeast cells used for production of ethanol is well developed and skills of artisans high, the specification fails to provide enough guidance as to how to make the claimed transformant, because expressing or overexpressing the genes encoding a)-j) does not always results in an increase of ethanol production. For example, overexpression of glutamate dehydrogenase may lead to decrease in ethanol production; see, for example, Lincino Da Silva et al. 1992. Overexpressing of glycerol 3-phosphate dehydrogenase results in drop of ethanol production; see for example Eglington J. M. et al, Yeast, 2002, and deletion of glycerol 3-phosphate dehydrogenase gene causes an increase in ethanol production (Valadi et al. 1998), which is the opposite of that what may expected from reading the disclosure. Deleting NADPH-dependent glutamate dehydrogenase, which is glutamate dehydrogenase, as recited in claims 43 and 57, causes not a decrease but and increase in ethanol production, which is the opposite of that what one would expect reading the claims; see Michnick et al. 1997(in IDS) and Nissen T. L. et al. 2000. All articles mentioned in this comment are enclosed in examiner's references, PTO Form 982. Furthermore, most of dehydrogenases are in forms that are NAD or NADP dependent and are involved in reactions in which substrate S are definite but X,Y and Z may differ. In summary, result of transformation will be a combination of the intricacies of the metabolism of the yeast cell selected for transformation and the form of dehydrogenase chosen, and only the combination of the genetic set of parental cell used for

transformation and the appropriate genes to be transferred leads to the desired result of increasing ethanol production, otherwise the probability of obtaining the claimed inventionis low. Thus, without further guidance on the part of Applicants regarding the parental cell to be transformed and the enzyme which is going to be expressed (overexpressed) in the transformant, probability of making the invention is low, and the experimentation left to those skilled in the art is extensive and undue

3. Conclusion

Claims 25 and 26 are allowed or reasons stated in the previous Office Actions. Applicants are advised that claims directed to the use of transformants of claim 25 and 26 contain allowable subject matter; for example the method of production of ethanol by the yeast transformants of claim 25 and 26 is fully described and enabled.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka whose telephone number is (571) 272-0944. The examiner can normally be reached on Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached on (571) 272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for

published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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